Issued April 22, 1913.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 166.

A. D. MELVIN, Chief of Bureau.

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BY

PHILIP RUPP, M. D., Ph. D.,
Chemist, Dairy Division.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., January 30, 1913.

Sir: I have the honor to transmit for publication as a bulletin of this bureau the accompanying manuscript, entitled "Chemical Changes Produced in Cows' Milk by Pasteurization," by Dr. Philip Rupp, chemist in the Dairy Division of this bureau. The paper describes a series of experiments made with the object of determining the effect of pasteurization at various temperatures on the chemical composition of cows' milk. The work was undertaken because of certain objections which are made against pasteurization, it having been asserted that the process injures the milk from a digestive and nutritive standpoint, particularly as a food for infants. It is satisfactory to note from the results of the work that, so far as the ordinary temperatures used in commercial pasteurization are concerned, these objections are not well founded.

The method of filtering the milk necessary for the carrying on of the tests consists of a new application by Dr. Rupp of the clay-cell filtration used by other scientists. This method is fully described and illustrated by a diagram.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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CHEMICAL CHANGES PRODUCED IN COWS' MILK BY PASTEURIZATION.

INTRODUCTION.

One of the objections frequently raised against pasteurized milk is that the heating produces changes in the chemical composition which make it unsuitable for infant nourishment. It is claimed that a portion of the soluble phosphate of lime becomes insoluble and that this change produces defective nutrition, while the changes in the lactalbumin and in the casein render the pasteurized milk more indigestible than raw milk, and therefore inferior as a food. These objections were brought forward at a time when high temperatures were employed in the sterilization of infants' milk, and they are made use of at the present day by the opponents of pasteurization, even though the temperatures used in pasteurizing are considerably lower.

The objects of the work herein described were to study the chemical changes produced by the heating of milk at the different temperatures used in commercial pasteurization and to determine whether a temperature that destroys all pathogenic germs can be used in pasteurization without having any appreciable effect on the chemical composition of the milk. Three points are considered: First, the changes in the phosphates are discussed in order to show to what extent the soluble phosphate of lime becomes insoluble. Second, the proteins are examined to determine to what extent the albumin is precipitated and the casein changed in its characteristic property of coagulating with rennin. Finally, the acidity of the milk is considered.

METHOD OF PASTEURIZATION EMPLOYED.

The milk was pasteurized in a constant-temperature bath. The water having reached the required temperature, the cool milk, in an Erlenmeyer flask closed with a valve to prevent evaporation, was placed in the water and occasionally moved about until it had acquired the temperature of the water. This usually required from 15 to 20 minutes, according to the height of the temperature. It was then allowed to remain for 30 minutes, after which it was rapidly cooled to about 12° C. (53° F.). The milk used was of very good quality and had a very low bacterial count. The variation in temperature during pasteurization was less than 0.5° C. (0.9° F.).

CHEMICAL CHANGES IN THE PHOSPHATES.

The vast majority of the experiments which show a diminution in the quantity of soluble calcium salts in heated milk as compared with raw milk refer to milk which has been heated to the boiling point. But few definite results can be found showing a diminution of the soluble calcium phosphate in pasteurized milk.

Diffloth[1] found a decrease in the soluble phosphates, amounting to a loss of 25.9 per cent, on heating milk to 60° C. (140° F.) for 30 minutes, while Babcock[2] believes that a change in the solubility of the lime salts is brought about when the milk is pasteurized, and bases his conclusion on the less ready coagulability of the milk by rennet.

In order to determine the effect of pasteurization on the solubility of the phosphates in the milk, the serum of both raw and pasteurized milk was examined.

METHOD OF FILTRATION.

A new application of the clay-cell filtration as used by Soxhlet[3] was employed. The clay cell of a galvanic element is thoroughly cleansed by boiling in dilute caustic soda and then washed by filtering hot water through the cell by suction. The cell is next boiled in dilute hydrochloric acid and again washed by suction with hot distilled water until the filtrate no longer gives a reaction for chlorin.

The method of filtration is easily understood by a description of the accompanying diagram (figure 1).

An inverted bell jar (*a*) (9 inches high and 4 inches in diameter) is covered with a perforated glass plate (*b*) holding a tube containing a plug of cotton to admit air. Inside the jar is a porous clay cell (*c*) of a galvanic element (5¼ inches high, 2⅛ inches in diameter, and the cell wall one-eighth of an inch thick) the opening of which is covered with a piece of rubber dam (*d*) of medium thickness and supported on a heavy glass plate (*e*). Both the rubber dam and the glass plate are perforated for the passage of a rubber stopper containing a glass tube with a Geissler stopcock (*f*). The upper end of the tube is flush with the glass plate *e*, while the lower end passes into a filtering flask (*g*) of 750 c. c. capacity. A glass tube with a two-way stopcock (*h*) is attached to the outlet of the flask, and this in turn is connected with the suction apparatus by means of a heavy rubber tube. In order to obtain airtight joints a little stopcock grease is placed on the glass plate, and the edge of the clay cell is also coated with a thin layer. The cover (*b*) of the bell jar is held down in the same manner.

---

[1] Diffloth, Paul. Du rôle de quelques agents physiques et chimiques dans l'insolubilisation des phosphates du lait. Bulletin des Sciences Pharmacologiques, vol. 10, p. 273–279. Paris, 1904. Abstract: Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, vol. 11, no. 7, p. 455–456. Berlin, Apr. 15, 1906.

[2] Babcock, S. M. The centrifugal separation of casein and insoluble phosphates from milk. Wisconsin Agricultural Experiment Station, Twelfth Annual Report (1895), p. 93–99. Madison, 1896.

[3] Soxhlet, Franz. Beiträge zur physiologischen Chemie der Milch. Journal für Praktische Chemie, new series, vol. 6, no. 1/2, p. 1–52. Leipsic, Aug. 24, 1873.

To begin filtration remove the cover, evacuate the apparatus, and while the suction is still on fill the bell-jar with milk. Then cut off the suction at *h*, replace the cover, and remove the suction tube. The apparatus is now ready to be placed in the refrigerator.

The advantage of this method over those previously employed is that the filtration can be continued indefinitely and the serum removed at any time without interfering with the filtration. Furthermore, no concentration of the serum can take place, as evaporation is excluded. The serum can be removed at any time by closing the stopcock *f*, admitting air by opening *h*, and removing the filtering flask. Then replace the flask, evacuate, close the stopcock *h*, and open *f*, and the apparatus is ready for further filtration.

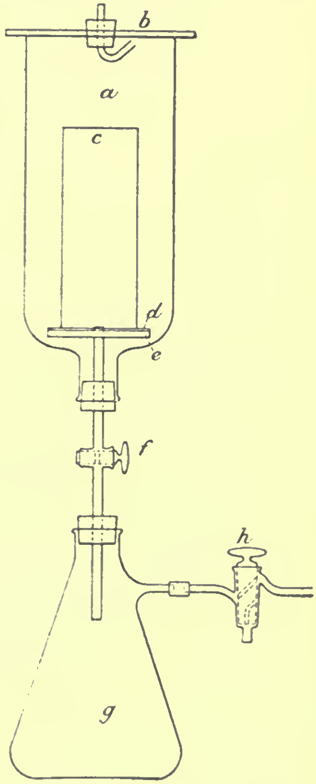

FIG. 1. Apparatus for filtering milk.

METHODS OF ANALYSIS.

Weigh about 40 grams of milk (equivalent to about 0.1 gram of phosphoric acid), evaporate to dryness, and ignite at a low red heat until the ash is free from carbon. Dissolve the ash in hot water containing a little hydrochloric acid and dilute to about 100 c. c.

Basic acetate precipitation.—Almost neutralize the solution of the ash with ammonia, add 2 c. c. of dilute acetic acid and a slight excess of ferric chlorid solution of known strength (7.5 c. c. of a 10 per cent solution). Now add 13 to 14 c. c. of a 10 per cent ammonium acetate solution, dilute to 350 to 400 c. c. and boil for about one-half to one minute, stirring occasionally to prevent bumping. If the quantity of ferric chlorid added was sufficient the precipitate will be brownish-red, and the precipitation will be complete as the boiling point is reached if the acetate was added in sufficient quantity. Filter boiling hot through two 12.5 cm. filters and wash immediately with a hot dilute ammonium acetate solution (one-half to one per cent), using a fine jet which is played around the edge of the precipitate, thus cutting it free from the paper in order to produce rapid filtration. Do not allow to drain dry. Wash the precipitate in this manner three to four times until free from chlorids, return to the original beaker with hot water, and dissolve by the addition of nitric acid. When the basic acetate precipitation is carried out as described above the filtration is always rapid and the precipitate never runs through the filter. In washing the precipitate with hot water, some flakes generally appear on concentrating the filtrate and washings. This, however, is not the case if a hot dilute solution of ammonium acetate is used.

The phosphoric acid in the basic acetate precipitate was determined by the molybdate method and weighed as magnesium pyrophosphate. The filtrate and washings from the basic acetate precipitation were concentrated and the calcium precipitated with ammonium oxalate. The oxalate was redissolved, reprecipitated, and weighed as calcium oxid. The filtrate and washings from calcium precipitation were evaporated, ammonium salts removed, and the magnesium determined as pyrophosphate.

For serum analysis evaporate about 70 grams and ignite at a dull red heat until the ash is free from carbon. In the basic acetate precipitation use 6 c. c. of ferric chlorid solution and 9 to 10 c. c. of ammonium acetate solution.

The casein phosphorus was determined by Neumann's method,[1] using, however, only 10 c. c. of concentrated sulphuric acid. The total phosphorus in the milk was also determined by Neumann's method.

The specific gravity was taken by means of a picnometer.

CHANGES IN THE COMPOSITION OF THE SERUM DURING FILTRATION.

De Vries and Boekhout,[2] using a Chamberland filter and pressure, found that the quantity of calcium in the serum did not vary in different fractions during the filtration, the first 20 c. c. of serum containing the same quantity of calcium as the second and third. In order to determine whether the serum remains of constant composition during filtration and for what length of time milk can be filtered at a temperature of 5° to 8° C. (41° to 46. 4° F.) without changing its composition, skim milk was filtered and the serum analyzed during a period of two weeks. Skim milk was used for this experiment because it filters more rapidly than whole milk. The results are shown in Table 1.

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[1] Neumann, Albert. Einfache Veraschungsmethode (Säuregenusch-Verasehung). Zeitschrift für Physiologische Chemie, vol. 37, no. 2, p. 115–142. Strasburg, Dec. 20, 1902.

[2] Ott de Vries, J. J., and Boekhout, F. W. J. Beitrag zur Kenntnis der Labgerinnung. Die Landwirtschaftlichen Versuchs-Stationen, vol. 55, no. 3, p. 221–239. Berlin, Feb. 12, 1901.

TABLE 1.—*Quantity of soluble phosphorus in the serum on different days during the filtration of raw skim milk, separated at 30° C. (86° F.).*

	Quantity.	Specific gravity.	Acidity.[1]	P_2O_5.	CaO.	MgO.
	Grams.			*Per cent.*	*Per cent.*	*Per cent.*
Casein				0.0638		
Raw skim milk		1.034	17.8	.2352	0.1671	0.0185
Serum:						
First day—3 hours	34.3	1.023		.0753	.0450	.0133
First day—next 18 hours	81.5	1.025	8.8	.1029	.0554	.0133
Second day	109.8	1.025	9.0	.1054	.0581	.0134
Do				.1055	.0588	.0133
Third day	109.3	1.025	9.0	.1055	.0578	.0127
Fourth day	112.0	1.025	9.0	.1048	.0585	.0127
Fifth day	105.7	1.025	9.0	.1054	.0589	.0130
Sixth and seventh days	205.0	1.025	9.0	.1057	.0579	.0129
Do				.1053	.0582	.0131
Tenth and eleventh days	155.0	1.025	10.0	.1074	.0609	.0131
Thirteenth and fourteenth days	158.5	1.025	12.0	.1082	.0633	.0139
Fifteenth and part of sixteenth day	105.0		14.6	.1110	.0683	.0145

[1] Number of cubic centimeters of $\frac{N}{10}$ alkali required to neutralize 100 c. c. of serum.

As will be seen from the above table there is an absorption of both phosphoric acid and calcium at the beginning of filtration, while the magnesium passes completely through the cell wall. For this reason the first portion of the serum must be rejected as worthless for making comparisons. The quantity to be rejected varies with the size of the cell used and may amount to from 60 to 90 c. c.

The specific gravity of the serum collected during the first 3 hours was 1.023, and it increased to 1.025 during the next 18 hours, after which time it remained constant during the remainder of the experiment.

The acidity was 8.8 on the first day and 9 on the second day. After the ninth day the acidity began to increase gradually, being 10 on the tenth and eleventh days, while on the fifteenth day it was 14.6, showing that the lactic acid had considerably increased.

The phosphoric acid during the first 3 hours amounted to only 0.0753 per cent, and it increased to 0.1029 per cent during the next 18 hours. On the second day it was 0.1054 per cent, and then remained constant until the tenth and eleventh days, when it began gradually to increase, amounting to 0.1110 per cent on the fifteenth day.

The calcium content was also low during the first 3 hours of filtration, amounting to 0.045 per cent, and it increased to 0.0554 per cent during the next 18 hours. On the second day it was 0.0581 per cent, and began to increase gradually as the acidity increased, amounting to 0.0683 per cent on the fifteenth day.

The magnesium remained constant during the first 11 days and then increased very slowly up to the fifteenth day.

The experiment shows, furthermore, that the filtration of a clean milk with low bacterial count can be continued for about one week without any appreciable chemical changes taking place in the serum, provided the temperature be kept at 6° to 8° C. (42.8° to 46.4° F.).

Before proceeding to the filtration of raw milk in the refrigerator, several experiments were made in which formaldehyde had been added to the milk as a preservative. This was done in order to carry on the filtration at room temperature. The results obtained showed that the serum, after the first day's filtration, remained of constant composition, and led up to carrying out the filtration of a pure milk in the refrigerator.

Having thus shown that the serum remains of constant composition after the first day of filtration, the next step was to determine whether there were any demonstrable changes produced in the soluble phosphates by pasteurization. In order to produce a maximum precipitation the highest temperature, 68.3° C. (155° F.), employed in the holder process was chosen. The results obtained are shown in Table 2.

TABLE 2.—*Quantity of soluble phosphates in the serum of raw whole milk and in whole milk pasteurized at 68.3° C. (155° F.) for 30 minutes.*

	Specific gravity.	Acidity.	P_2O_5.	CaO.	MgO.
			Per cent.	*Per cent.*	*Per cent.*
Casein			0.0595		
Raw whole milk	1.029	18.0	.2219	0.1749	0.0203
Raw serum:					
Second and third days	1.026	9.8	.1074	.0625	.0142
Do			.1078	.0624	.0142
Fifth and sixth days	1.026	9.6	.1082	.0631	.0143
Pasteurized serum:					
Second and third days	1.026	9.6	.1078	.0611	.0140
Do			.1063	.0613	.0140
Fifth and sixth days	1.026	9.4	.1089	.0627	.0141

It will be seen from the table that the changes produced by pasteurization at 68.3° C. (155° F.) for 30 minutes are trifling. The phosphoric acid content remains the same in both the raw and the pasteurized serum. The quantity of lime salts, while a trifle lower in the pasteurized than in the raw serum, is still within the limit of error, and the magnesia remains the same in both serums.

Other experiments made with skim milk showed results agreeing in every particular with this one made with whole milk. All preliminary experiments were made with skim milk because, as previously stated, it filters more readily and thus gives a larger quantity of serum for analysis.

Comparing Table 1 with Table 2 we find that the variation in the quantity of soluble lime salts between the two samples of milk is far greater than that found between the raw and pasteurized serums. For this reason the objection frequently raised against pasteurized milk as an infant food, on the ground that the soluble lime salts are diminished by the process of pasteurization, is without foundation.

CHEMICAL CHANGES IN THE PROTEINS.

LACTALBUMIN.

Different investigators do not agree as to the exact temperature at which the coagulation of the albumin begins. Steiner [1] found no change on heating milk for 25 minutes at 60° C. (140° F.), while Solomin [2] says that the change seems to begin at this temperature, and Jensen and Plattner [3] also found some coagulation in milk heated for five hours at 60° C. (140° F.). Babcock [4] could find no change on heating milk for 20 minutes at 65° C. (149° F.), while Woll [5] states that coagulation begins at this temperature. According to Willoughby [6] the change begins at 70° C. (158° F.), while de Freudenreich [7] found 15 to 20 per cent coagulated on heating milk for 30 minutes at 68° to 69.5° C. (154.4° to 157° F.). A series of experiments were therefore undertaken to determine at what temperature the change in the albumin begins, and to what extent it is coagulated at the different temperatures commonly employed in the pasteurization of milk.

METHODS OF ANALYSIS.

Casein.—To 10 grams of milk were added 50 c. c. of distilled water at 40° C. (104° F.), then 1.5 to 2 c. c. of a saturated alum solution were added gradually. The precipitate was allowed to settle, was then filtered and washed. The precipitate and filter paper were treated by the Gunning method, adding about 0.2 gram of copper sulphate.

Albumin and globulin.—The albumin and globulin in the filtrate and washings were precipitated with 10 c. c. of Almén's tannin solution; this was allowed to settle, was then filtered and washed. The precipitate was treated the same as under casein.

Tables 3 to 6 show the results of the experiments to determine the lactalbumin precipitated in whole milk pasteurized for 30 minutes at temperatures ranging from 62.8° C. (145° F.) to 71.1° C. (160 F.).

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[1] Steiner, R. Beiträge zur Kenntnis des Einflusses der Pasteurisierung auf die Beschaffenheit der Milch und auf den Butterungs Prozesz. Milch-Zeitung, vol. 30, no. 26, p. 401–403, June 29; no. 28, p. 435, July 13. Leipsic, 1901.

[2] Solomin, P. Ueber die beim Erhitzen der Milch ausfallenden Eiweissmengen. Archiv für Hygiene, vol. 28, no. 1, p. 43–48. Munich and Leipsic, 1896.

[3] Jensen, Orla, and Plattner, Ernest. De l'action du chauffage sur le lait de vache. Revue Générale du Lait, vol. 4, no. 16, p. 361–368, May 30; no. 17, p. 388–397, June 15; no. 18, p. 419–424. Lierre, 1905.

[4] Babcock, S. M. The centrifugal separation of casein and insoluble phosphates from milk. Wisconsin Agricultural Experiment Station, Twelfth Annual Report (1895), p. 93–99. Madison, 1896.

[5] Woll, F. W. The effect of pasteurization and sterilization on the viscosity and fat globules of milk and cream. Wisconsin Agricultural Experiment Station, Twelfth Annual Report (1895), p. 164–173. Madison, 1896.

[6] Willoughby, Edward F. Milk, its production and uses. London, 1903. See p. 125–126.

[7] De Freudenreich, Ed. Sur la pasteurisation du lait dans l'alimentation de l'enfance. Revue Générale du Lait, vol. 4, no. 19, p. 433–437. Lierre, July 15, 1905.

TABLE 3.—*Quantity of albumin precipitated in raw whole milk and in milk pasteurized at 62.8° C. (145° F.) for 30 minutes.*

	Raw milk.			Pasteurized milk.		
	I.	II.	Average.	I.	II.	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Casein nitrogen	0.4003	0.4027	0.4015	0.4015	0.4010	0.4013
Albumin+globulin nitrogen	.0996	.0998	.0997	.0996	.0997	.0997
PROTEIN=NITROGEN×6.38.						
Casein	2.554	2.569	2.562	2.561	2.558	2.560
Albumin+globulin	.635	.637	.636	.635	.636	.636

No albumin precipitated.

TABLE 4.—*Quantity of albumin precipitated in raw whole milk and in milk pasteurized at 65.6° C. (150° F.) for 30 minutes.*

	Raw milk.			Pasteurized milk.		
	I.	II.	Average.	I.	II.	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Casein nitrogen	0.4260	0.4218	0.4239	0.4320	0.4328	0.4324
Albumin+globulin nitrogen	.0983	.0978	.0981	.0920	.0929	.0925
PROTEIN=NITROGEN×6.38.						
Casein	2.717	2.691	2.704	2.756	2.762	2.759
Albumin+globulin	.627	.624	.626	.587	.593	.590

Albumin precipitated=5.75 per cent.

TABLE 5.—*Quantity of albumin precipitated in raw whole milk and in milk pasteurized at 68.3° C. (155° F.) for 30 minutes.*

	Raw milk.			Pasteurized milk.		
	I.	II.	Average.	I.	II.	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Casein nitrogen	0.3739	0.3812	0.3776	0.3910	0.3878	0.3894
Albumin+globulin nitrogen	.0963	.0981	.0972	.0863	.0833	.0848
PROTEIN=NITROGEN×6.38.						
Casein	2.385	2.432	2.409	2.495	2.474	2.485
Albumin+globulin	.614	.626	.620	.551	.531	.541

Albumin precipitated=12.75 per cent.

TABLE 6.—*Quantity of albumin precipitated in raw whole milk and in milk pasteurized at 71.1° C. (160° F.) for 30 minutes.*

	Raw milk.			Pasteurized milk.		
	I.	II.	Average.	I.	II.	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Casein nitrogen	0.3860	0.3851	0.3856	0.4200	0.4169	0.4185
Albumin+globulin nitrogen	.0961	.0989	.0975	.0681	.0667	.0674
PROTEIN=NITROGEN×6.38.						
Casein	2.462	2.457	2.460	2.679	2.660	2.670
Albumin+globulin	.613	.631	.622	.434	.426	.430

Albumin precipitated=30.87 per cent.

From an analysis of the above tables it is apparent that no albumin is coagulated on heating milk for 30 minutes at 62.8° C. (145° F.), the temperature most commonly used in the holder process in commercial pasteurization. At 65.6° C. (150° F.) the separation of the albumin has begun, 5.71 per cent having become insoluble. At 68.3° C. (155° F.) the quantity has increased to 12.76 per cent, while at 71.1° C. (160° F.) 30.87 per cent of the albumin has been coagulated.

CASEIN.

The condition in which casein exists in milk has not as yet been fully explained. The effect of heat shows itself in two ways. In the first place, the rennin coagulation may be accelerated, retarded, or even inhibited; and in the second place, the coagulum may assume a different character from that obtained from raw milk.

The great majority of authors agree that no change in rennin coagulation takes place below 65° C. (149° F.). Steiner [1] found a retardation in milk heated to 60° C. (140° F.), while Fleischmann and Morgen [2] found the milk less sensitive to rennin when heated for two hours from 60° to 70° C. (140° to 158° F.). According to Van Slyke and Publow [3] milk heated above 65.6° C. (150° F.) for a considerable length of time coagulates less rapidly and the coagulum is highly flocculent. De Freudenreich [1] finds the coagulum unchanged at 68° to 69.5° C. (154.4° to 157.1° F.). Jensen and Plattner [1] state that retardation begins on heating milk for five hours at 70° C. (158° F.), while Mayer [4] places the beginning at 75° C. (167° F.). Stassano and

[1] Loc. cit.

[2] Fleischmann, W., and Morgen, A. Einiges über die nach Scherff's Verfahren conservirte Flaschen milch. Die Landwirthschaftlichen Versuch Stationen, vol. 28, p. 321 332. Berlin, 1883.

[3] Van Slyke, Lucius L., and Publow, Charles A. The science and practice of cheese-making, p. 310. New York, 1909.

[4] Mayer, Adolf. Bestimmungen der Wirksamkeit des Labfermentes unter verschiedenen äuszeren Umständen. Milch-Zeitung, vol. 10, no. 2, p. 17-19, Jan. 12; no. 3, p. 33-38, Jan. 19; no. 4, p. 49-52, Jan. 26; no. 6, p. 81-84, Feb. 9. Bremen, 1881.

Talarico [1] differ from all the preceding in that they observed an acceleration of rennin coagulation in milk heated from 55° to 65° C. (131° to 149° F.), while at 70° C. (158° F.) the heated milk behaved the same as raw milk, and above 70° C. (158° F.) the retardation began.

The following experiments were made with raw milk and milk pasteurized at various temperatures:

METHOD OF COAGULATION.

The apparatus consisted of a large galvanized-iron box having four cylindrical compartments surrounded by water. The upper openings were of sufficient size to admit a cylindrical percolater (1 foot high and 2½ inches in diameter), the lower openings being about one-fourth of an inch in diameter. A small glass tube drawn out into a capillary was inserted into the neck of the percolator by means of a perforated rubber stopper.

In making the test, the water in the box was heated to the required temperature, the percolators were then placed in the compartments and allowed to remain until they had acquired the temperature of the bath, the upper openings being covered. Next 200 c. c. of milk were heated, the rennin solution added, and, after mixing, poured into the percolator. The upper opening was then closed with a cover and the time required for the milk to coagulate in the tube noted. The flow through the tube was regulated by breaking off the capillary to such an extent that all tubes dropped at the same rate.

For each experiment 200 c. c. of milk were heated in an Erlenmeyer flask to 35° C. (95° F.) and 5 c. c. of rennin solution (0.15: 100 c. c. of water) added. This was taken as the beginning of the coagulation time; the end point was when the milk stopped dropping from the capillary tube. The rennin solution was made of such strength that the raw milk coagulated in about 20 minutes. The results are shown in the following table:

TABLE 7.—*Time required for rennin coagulation of raw whole milk and of whole milk pasteurized at different temperatures. Milk 200 c. c., rennin solution (0.15 gram: 100 c. c. of water) 5 c. c.*

Experiment.	Raw milk.	Milk pasteurized at—				
		55° C. (131° F.).	60° C. (140° F.).	65° C. (149° F.).	70° C. (158° F.).	75° C. (167° F.).
	M. s.	*M. s.*	*M. s.*	*M. s.*	*M. s.*	*M. s.*
I	18 30	17 28	17 10	17 12	....	....
	18 08	16 56	16 53	17 12	....	....
II	19 34	....	....	....	20 38	36 30
	19 23	....	....	....	20 25	37 30

[1] Stassano, H., and Talarico, J. De l'influence de la cuisson sur la caséification du lait par le lab-ferment. Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie, vol. 69 (année 62, tome 2), no. 28, p. 254-255. Paris, Aug. 5, 1910.

From the above table it will be seen that milk pasteurized at 55° C. (131° F.), 60° C. (140° F.), and 65° C. (149° F.) all coagulate more rapidly with rennin than does the raw milk. This result was obtained in all cases in a large number of experiments. At 70° C. (158° F.) the retardation of rennin coagulation has begun, while at 75° C. (167° F.) the time has about doubled and the coagulum is highly flocculent.

CHANGES IN THE ACIDITY.

Woll[1] and de Freudenreich[1] find no appreciable change in the acidity of milk pasteurized at 67° to 68° C. (153° to 154.4° F.) for 20 minutes. Höft[2] and Jensen and Plattner[1] on the other hand find a diminution in the acidity on heating milk.

From a large number of experiments made during the coagulation of milk by rennin, it was found that the acidity of the milk always diminished during pasteurization. While the diminution was slight it amounted to several tenths of a cubic centimeter, and was always sufficient to be determined by titration with tenth-normal caustic-soda solution. The diminution, however, is insignificant and is probably due to a loss of carbonic acid during the heating. Furthermore, the diminution varies in different samples of milk.

SUMMARY AND CONCLUSIONS.

1. Milk pasteurized by the holder process at 62.8° C. (145° F.) for 30 minutes does not undergo any appreciable chemical change.

2. The soluble phosphates of lime and magnesia do not become insoluble. At 68.3° C. (155° F.) the quantity of phosphoric acid, lime, and magnesia in the serum of both raw and pasteurized milk are practically the same.

3. The albumin does not coagulate at 62.8° C. (145° F.), but at 65.6° C. (150° F.) 5.75 per cent of the albumin is rendered insoluble. As the temperature increases the amount of coagulated albumin increases. At 68.3° C. (155° F.) the quantity increases to 12.75 per cent, and at 71.1° C. (160° F.) it amounts to 30.78 per cent.

4. The time required for coagulating the casein by rennin is slightly less in milk pasteurized at temperatures up to 65° C. (149° F.) than it is in raw milk. At 70° C. (158° F.) there is a slight retardation, while at 75° C. (167° F.) the time has almost doubled.

5. The acidity as determined by titration is slightly diminished in pasteurized milk.

[1] Loc. cit.

[2] Höft, H. Ueber die Veränderung der Acidität der Milch beim Erhitzen. Milch-Zeitung, vol. 30, no. 7, p. 103. Leipzig, Feb. 16, 1901.